AMENDMENT NO. 1 DECEMBER 2014
TO
IS 13424 : 2001 SAFETY EVALUATION OF BATHING BARS AND TOILET SOAPS — METHODS OF TEST

(First Revision)

(*Second cover page*, *Foreword*, *paras* 2, 3 *and* 4) — Substitute the following for the existing paras:

'Detergent products which come in contact with skin should be free from skin sensitizing/skin allergy producing components. Such components even when present in minute levels (ppm — part per million concentration) in the formulation, would cause itching, redness, swelling and blister formation on the skin. The onset of such episodes is usually a delayed response from the time of first contact with the product which makes it difficult to identify the incriminating product/ingredient, even by an experienced medical expert. The detection involves cumbersome procedure of patch testing on skin with all suspect materials on people who are susceptible to such skin allergy. The intractable nature of this skin condition may cause unnecessary hardships to the consumers. Hence, it is essential that manufacturers of detergent products make sure that their products are free from such hazard, by subjecting their products to safety evaluation according to the test methods stipulated in this standard.

Whereas products which contain novel ingredients, prior to initiating the test involving novel ingredients or products containing novel ingredients, preliminary safety assessment using Quantitative Risk Assessment (QRA) tools and/or non-animal methods followed by Skin Irritation Test (Patch Test in humans) should be carried out.

The manufacturer of bathing bars and toilet soaps containing novel ingredients may submit the safety evaluation data based on alternative non-animal test methods for skin sensitization test to the concerned State Licencing Authorities for their consideration and approval.

Human Repeated Insult Patch Test (HRIPT) is not a predictive skin sensitization test method for unknown materials. It is a confirmatory test for known materials.'

(*Second cover page*, *Foreword*, *para* 6) — Substitute the following for the existing para:

'All testing whether involving human subjects is to comply with the legislative/regulatory requirements with reference to testing on volunteers. Where safety testing involves human participants, approval shall be obtained from Institutional Ethics Committee before initiating the study.'

(*Page* 1, *clause* **1**) — Substitute the following for the existing clause:

'1 SCOPE

This standard prescribes a short duration skin irritancy test and skin sensitization tests on human subjects.'

(*Page* 2, *clauses* **3** *to* **3.2.5**) — Substitute the following for the existing clauses:

'3 SKIN SENSITIZATION TEST ON HUMAN SUBJECTS — HUMAN REPEATED INSULT PATCH TEST (HRIPT)

3.1 General

This test is based on the principle that in an allergic individual, the whole skin is capable of reacting with the allergen. Therefore, if the substance is applied on the skin on any part of the body, it will result in dermatitis at the site. Although this test is simple, yet a little experience is essential for accurate interpretation of results.

3.2 Instructions

3.2.1 Prior to initiating the test involving novel ingredients or products containing novel ingredients, preliminary safety assessment using Quantitative Risk Assessment (QRA) tools and/or non-animal methods followed by Skin Irritation Test (Patch Test in humans) should be carried out.

Price Group 3

3.2.2 The test should not be applied, if the volunteer is already having acute dermatitis because there is risk of aggravation of the dermatitis and occurrence of false positive reactions. The test should also be postponed, if the volunteer is being given systemic corticosteroid or other cyclophosphamide, methotrexate, azathioprine, etc, because these drugs can suppress the reaction and lead to false negative results. Further, it should be ensured that the volunteer is not under any form of medication immediately before or during the study, which might impact the outcome of the study; in such case, the volunteer should be excluded or dropped from the study.

3.2.3 The substance to be used as allergen for human repeat insult patch test may be either the finished product as such or preferably each of the suspect ingredient used in the product.

3.2.4 When using ingredients, it is important to use each agent in a specified concentration and also in a specified vehicle (or base). This is necessary to avoid false positive or false negative results, because if the concentration of the agent is too high, it may result in irritant dermatitis at the test site, while if the concentration is too low, it may not evoke any reaction. Similarly, if the vehicle used for dissolving the agent does not allow the agent to penetrate the epidermal barrier, it may result in a false negative reaction. In the case of cosmetic, it will be especially useful if agents which may be used as substitutes of the standard ingredients are also included for patch tests. This shall help the industry to know the substitutes which may be employed in the manufacture of the cosmetic in place of the ingredient causing contact dermatitis.

3.2.5 Normal saline (0.9 percent aqueous solution of sodium chloride) or any other agent(s) used as a vehicle for dissolving the ingredient should also be applied as controls for the human repeated insult patch test. It is necessary to ensure that the controls do not produce any reaction at the site of patch test.

3.3 Procedure

3.3.1 Apply the neat product as such (or diluted solution as given in Table 2) on the upper arms or back of human subjects as follows:

a) The skin should essentially be non-hairy and free from any type of skin lesions or other dermatological problems. In case the back is hairy, the hair can be cut off by clipper or shaver before applying the patches, taking care to avoid any abrasions, cuts or lesions.
b) In case of rinse off products, rinse the treated sites with water to remove any residue after each patch removal (or the specified time of contact in case of open patch).
c) If the volunteer experiences unbearable discomfort with any of the patches, the volunteer is instructed to remove such patches any time prior to the targeted 4 h contact (where applicable). Mark such sites with a blue/ black skin marker to facilitate evaluation later.
d) The volunteer is also requested to note down the sign and symptoms of the discomfort and the time of removal of the patch and hand it over to the investigator.

Table 2 Method of Product Application

(*Clause* 3.3.1)

Sl No. (1)	Type of Patch (2)	Products (3)
i)	Open	Relevant bathing bar and toilet soap
ii)	Semi occluded (keeps open for up to half an hour post application and then occlude.)	Relevant bathing bar and toilet soap
iii)	Occluded	Relevant bathing bar and toilet soap
iv)	Short duration open patch test (30 min)	Relevant bathing bar and toilet soap

3.3.1.1 *Inclusion and exclusion criteria for safety evaluation*

a) *Inclusion criteria*:

1) Voluntary men/women between 18 and 65 years;
2) Photo type III to V;
3) Having apparently healthy skin on test area;
4) For whom the investigator considers that the compliance will be correct;

5) Co-operating, informed of the need and duration of the examinations and ready to comply with protocol procedures;
6) Having signed a consent form;
7) Willingness to avoid intense UV exposure on test site (sun or artificial UV), during the course of the study;
8) Willingness to avoid excessive water contact (for example, swimming, etc) or activity which causes excessive sweating (for example, exercise, sauna etc..), during the course of the study;
9) Should be able to read and write (in English, Hindi or local language); and
10) Having valid proof of identity and age.

b) *Exclusion criteria*:

1) Pregnant/nursing mothers;
2) Scars, excessive terminal hair or tattoo on the study area;
3) Henna tattoo anywhere on the body (in case of studies involving hair dyes);
4) Dermatological infection/pathology on the level of study area;
5) Hypersensitivity, allergy antecedent (to any cosmetic product, raw material or hair dye);
6) Any clinically significant systemic or cutaneous disease, which may interfere with study treatment or procedures;
7) Chronic illness which may influence the outcome of the study;
8) Subjects on any medical treatment either systemic or topical which may interfere with the performance of the study treatment (presently or in the past 1 month); and
9) Subject in an exclusion period or participating in another food, cosmetic or therapeutic trial.

3.3.2 Select 200 healthy adult subjects, preferably equal number of males and females who do not have any previous history of adverse skin conditions or allergic reaction and are not under medication likely to interfere with the results. Pregnant ladies and breast feeding mothers should be excluded. Explain the test procedure to volunteers and obtain a signed informed consent from each of them**.**

3.3.3 Ideally use ready-made standard test patches measuring about 1 cm diameter or 1 sq. cm. Take 0.04 ml or 0.04 g of the sample using a micropipette or weighing balance (as applicable) on the patch and apply the patch on the upper arm or back as mentioned in **3.3.1**. Alternatively, if such patches are not available, use 1 cm diameter discs made out of chromatography paper (Whatman No. 3) taken on a slightly bigger polythene sheet having about 0.25 cm hole punched at the centre and fixed on the adhesive tape. Keep about 2.5 cm distance between the two adjacent test patches (filter paper discs).

3.3.3.1 *Testing with volatile substances for sample application*

3.3.3.1.1 Volatile substances can act as primary irritant, if test by the standard occluded patch test technique. Such agents should, therefore, be tested by the cup technique. In this method, the volatile agent is soaked into a small piece of absorbent cotton filter paper which is placed at the bottom of specially designed (*see* Fig.1). This cup is inverted on the forcarm-skin of the individual and bandaged. Care should be taken by ensure that the piece of filter paper remains sticking to the bottom of the cup and does not fall on the skin and the skin is exposed only to the vapours of the agent.

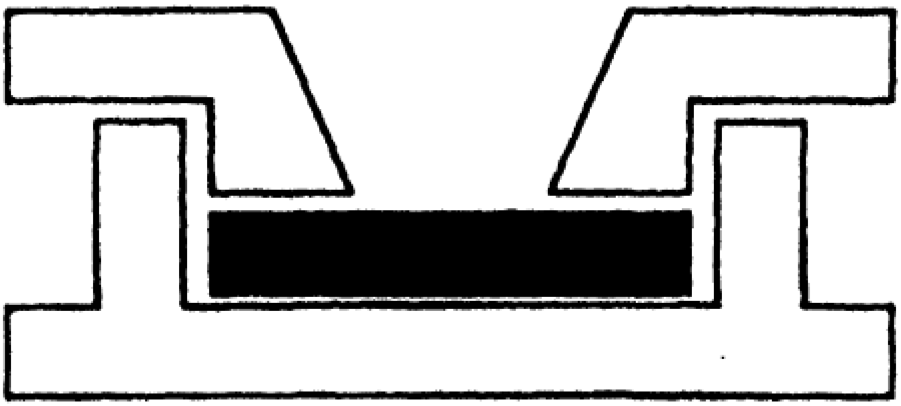

Fig. 1 Antigen Cup

3.3.3.1.2 If a finished product containing volatile substances is to be tested, the agent should be painted on the skin and left exposed for 30 min to allow the volatile substance to evaporate. After this, the area is occluded as for the standards patch test.

3.3.4 Various allergens should be incorporated into different patches and all these patches should be applied on the skin of the volunteer so that the allergen is in direct contact with the skin. Various patches should be numbered and an accurate account of the allergens put on these patches should be entered into a register for records.

3.4 Timing and Location of the Patch Test

3.4.1 *Induction Phase*

There is first induction phase where patches as described above, shall be applied on thrice a week basis for three weeks (single patch of the sample on each volunteer). The patch is removed after 24 h of contact each time and clinical examination is carried out 24 h after patch removal (or 24 h after product contact in the case of open patches). In case the original site shows a reaction of >2 on the erythema scale, then an adjacent area shall be patched.

3.4.2 *Rest Phase*

After the three week (21 days) induction phase, there shall be no more patch application, this is called as rest phase. The rest phase involves two weeks (14 days) of no application of any patch in order to develop (if at all) sensitization to the test product.

3.4.3 *Challenge Phase*

During challenge phase, single patch for each product or ingredient (applied during induction phase) will be put on a new site on upper arm or back (naïve skin site) at approximately a similar position.

Patch is removed at 24 h and scoring done at 24 h, 48 h, and 72 h after removal [or 24 h, 48 h, and 72 h after product contact in the case of open patches (120 h optional)].

3.5 Scales for Evaluation

3.5.1 The Draize scale (erythema and oedema) as given in Table 3 is used for evaluation during induction phase.

Table 3 Draize Scale for Scoring the Treatment Sites
(*Clause* 3.5.1)

Sl No. (1)	Score for Erythema/ Dryness/ Wrinkles (2)	Reaction (3)	Score for Oedema (4)	Reaction (5)
i)	0	No reaction	0	No reaction
ii)	1	Very slight erythema/dryness with shiny appearance	1	Very slight oedema
iii)	2	Slight erythema/ dryness/ wrinkles	2	Slight oedema
iv)	3	Moderate erythema/ dryness/ wrinkle	3	Moderate oedema
v)	4	Severe erythema/ wrinkle/ scale	4	Severe oedema

3.5.2 The international Contact Dermatitis Research Group Scale (ICDRG Scale) as given in Table 4 is used for evaluation during challenge phase.

Table 4 International Contact Dermatitis Research Group Scale (ICDRG Scale) for Scoring the Treatment Sites

(*Clause* 3.5.2)

Sl No. (1)	Symbol (2)	Morphology (3)	Interpretation (4)
i)	-	No reaction	Negative
ii)	?	Erythema only, no infiltration	Doubtful reaction
iii)	+	Erythema, infiltration, possibly discrete papules	Weak positive reaction
iv)	++	Erythema, infiltration, papules, vesicles	Strong positive reaction
v)	+++	Erythema, infiltration, confluent vesicles	Extreme positive reaction
vi)	ir	Different types of reactions (soap effect, vesicles, blister, necrosis)	Irritant reaction
7	Nt	—	Not tested

3.6 Interpretation of Score

3.6.1 Reactions produced by solid substances are generally milder, as the amount of the allergen that can enter the skin is usually much less. Thus even milder reactions produced by solid agents should be considered significant.

3.6.2 Sometimes, when the hypersensitivity in a volunteer is very mild, there may be no reaction at the patch test site after 48 h, though after an additional 24 h, there may be evidence of dermatitis. It is, therefore, preferable to take another reading after approximately 72 h.

3.6.3 Reaction during induction phase suggests skin irritation or pre-existing sensitization to the test material. Prominent reactions in the challenge phase suggest allergenicity to the ingredients of the test material.

3.6.4 Some individuals develop dermatitis reaction to the adhesive plaster which may spread to involve the entire test site, making it difficult to observe any reaction to the allergen. In such an instance the patch can be applied on the upper arm using only small strips of adhesive plaster or suitable hypo allergenic plaster.

3.6.5 Sometimes particularly in summer, the volunteer may develop a miliaria (prickly heat) - like reaction at the patch test site due to occlusion. This reaction is not reproducible and, therefore, it should be regarded as false positive reaction.

3.6.6 Some chemicals particularly nickel sulphate can at times lead to a pustular reaction which also is non-specific**.**

3.7 Report of Result

The report of a Human Repeat Insult Patch Test shall mention the following data:

a) Concentration of the chemical/ agent;
b) Amount used;
c) Areas of skin contacted;
d) Site of application;
e) Number of days the patch was left on the skin; and
f) Period after removal of the patch that the readings were made.

(*Page* 4, *clause* **4**) **—** Delete.

(CHD 25)

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